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A New Extractive Spectrophotometric Method for the Estimation of Alosetron

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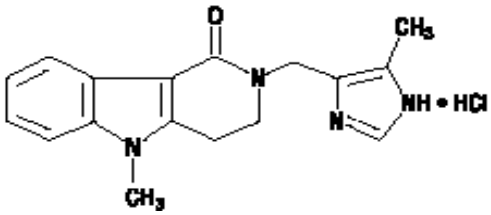
ABSTRACT

Two simple, accurate, rapid and sensitive Methods (A and B) have been developed for the estimation of Alosetron in its pharmaceutical dosage form. The Method A is based on the formation yellow colored chromogen, due to reaction of Alosetron Hydrochloride with Metanil yellow dye, formation of ion association complexes of the drug with dyes in phosphate buffer of pH 3.6 followed by their extraction in chloroform which exhibits λ_{max} at 410 nm. The Method B is based on the formation of light yellow colored chromogen due to reaction of Alosetron Hydrochloride with Methyl Orange dye, formation of ion association complexes of the drug with dyes in phosphate buffer of pH 3.6 followed by their extraction in chloroform, which exhibits λ_{max} at 422 nm. The absorbance-concentration plot is linear over the range of 5-60 mcg/mL for Method A and 50-120 mcg/mL for Method B. Results of analysis for all the methods were validated statistically and by recovery studies. The proposed methods are precise, accurate, economical and sensitive for the estimation of Alosetron in bulk drug and in its Tablet dosage form.

INTRODUCTION

Alosetron HCl (AST), (Merck Index, 2001) (Lotronex) chemically is designated as 2, 3, 4, 5-tetrahydro-5-methyl-2-[(5-methyl-1H-imidazol-4-yl)methyl]-1H-pyrido[4,3-b]indol-1-one, Monohydrochloride. Alosetron is achiral and has the empirical formula: $C_{17}H_{18}N_4O.HCl$, representing a molecular weight: 330.8 gms/mol.

It is a potent and selective antagonist of the serotonin 5-HT_3 receptor type. Activation of these receptors affects the regulation of visceral pain, colonic transit, and GI secretions. By blocking the action of serotonin on the intestinal system, the receptors are able to effectively control Irritable Bowel Syndrome (IBS). This reduces the cramping, stomach pain, stomach discomfort, urgency, and diarrhea caused by IBS. 5-HT_3 receptors are nonselective cation channels that are extensively distributed on enteric neurons in the human gastrointestinal tract, as well as other peripheral and central locations. AST inhibit activation of non-selective cation channels, results in the modulation of the enteric nervous system in neuronal depolarization affect. Literature survey reveals a few chromatographic methods (Evans *et al*., 2003, Jocic *et al*., 2009, Milton *et al*., 2002, Morgan *et al*., 1997) to determine the AST in tablet dosage form and also in biological fluids. No Spectrophotometric methods are reported. So far, no assay methods by liquid chromatography were reported for the estimation of AST in pharmaceutical dosage forms at the time of commencement of these investigations.



Structure of Alosetron Hydrocloride

EXPERIMENTAL WORK

Instrument

Elico double beam Ultra Violet- Visible double beam Spectrophotometer SL-244 with 1cm matched quartz cells was used for all spectral measurements.

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Preparation of Reagents

All chemicals used were of analytical reagent grade.

Preparation of Metanil Yellow (0.1% w/v)

Weigh 100 mg of metanil yellow accurately and transfer into a 100 mL volumetric flask and dissolved it by adding methanol and make up to the mark

Preparation of Methyl Orange

Weigh 100 mg of methyl orange accurately and transfer into a 100 mL volumetric flask and add 80 mL of water and add sufficient ethanol (95%) to produce 100 mL.

Preparation of Phosphate Buffer (3.6)

Dissolve 0.900 gm of anhydrous disodium phosphate and 1.298 gm of citric acid monohydrate in sufficient water to produce 1000 mL.

Preparation of standard solution of 100mg in 100ml stock solution

Weigh 100 mg of bulk drug (Alosetron Hydrochloride) and dissolve in water and make up to 100 mL to give a stock solution of 1 mg/mL.

ASSAY PROCEDURE

Method A

Aliquots of standard drug solution of Alosetron Hydrochloride (0.05 – 0.6 mL) of (100 mcg/mL) were taken and transferred into series of 100 mL separating funnels. To each funnel add 2 mL of buffer solution (pH 3.6) and 2 mL of Metanil Yellow was added. Then 5 mL of chloroform was added to each separating funnel and the contents were shaken for 2 minutes and allowed to separate. The absorbance of the solutions were measured at 410 nm against reagent blank, and the calibration curve was plotted. Similarly the absorbance of the sample solution was measured, and the amount of Alosetron Hydrochloride was determined by referring to the calibration curve.

Method B

Aliquots of standard drug solution of Alosetron Hydrochloride (0.5-1.2 mL) of (100 mcg/mL) were taken and transferred into series of 100 mL separating funnels. To each funnel add 1.5 mL of buffer solution (pH 3.6) and 2.5 mL of Methyl Orange was added. Then 5 mL of chloroform was added to each separating funnel and the contents were shaken for 2 minutes and allowed to separate. The absorbance of the solutions were measured at 422 nm against reagent blank, and the calibration curve was plotted. Similarly the absorbance of the sample solution was measured, and the amount of Alosetron Hydrochloride was determined by referring to the calibration curve.

Preparation of sample solution

20 tablets of Alosetron Hydrochloride (Lotronex, 0.562mg, GSK) were accurately weighed and powered. Tablet powder equivalent to 100 mg of Alosetron was dissolved in 5 mL of Water and made up to 100 mL with distilled water, sonicated for 15 min and filtered.

The solution was suitably diluted and analyzed as given under the assay procedure for bulk sample. The analysis procedure was repeated three times with Tablet formulations and the results of analysis for the method are shown in **Table: II**.

Recovery Studies

To ensure the accuracy and reproducibility of the results obtained, known concentration of the pure drug solution was added to the previously analyzed formulated solution samples and these samples were reanalyzed by the proposed method and also preformed recovery studies. The percentage recoveries, thus obtained for method is given in **Table: II.**

RESULTS AND DISCUSSIONS

The optimum conditions were established by varying one parameter at a time and keeping the others fixed and observing the effect on absorbance of chromogen.

In the present study, the Method A and B involves the formation of ion association complexes of the drug with dyes Metanil Yellow and Methyl Orange respectively with Phosphate buffer of pH 3.6 followed by their extraction in chloroform which gives yellow colored chromogens, having absorbance maximum at 410 nm and 422 nm respectively. Stability study of the developed chromogens were carried out by measuring the absorbance values at time intervals 15 min for 2 hrs, and the yellow colored chromogens were found to be stable for more than 2 hrs at room temperature. The linearity was found to be in the concentration range of 5-60 mcg/mL and 5-120 mcg/mL for Method A and B respectively.

Statistical analysis was carried out and the results were found to be satisfactory. Relative standard deviation values were low indicating the reproducibility of the proposed methods. Recovery studies were close to 100% that indicates the accuracy and precision of the proposed methods. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity, Sandell's sensitivity and other parameters are presented in **Table I.**

This new procedures for the spectrophotometric determination of alosetron hydrochloride described in this work is simple, rapid and cost-effective with high accuracy and precision, when compared with previously reported procedures. It could find application as a convenient technique for the in-process control analysis of alosetron hydrochloride in bulk and its pharmaceutical formulations.

Table. 1: Optical Characteristics and Precision Data.

Parameters	Method A	Method B
λ_{max} (nm)	410	422
Beer's law limits	5-60	50-120
Molar absorptivity (l/mol.cm)	$1.63X10^4$	$1.76X10^4$
Sand ell's sensitivity (micrograms/cm^2/0.001 absorbance unit)	0.0612	1.3953
Regression Equation* (Y)		
Slope (m)	0.013	0.005
Intercept (c)	0.215	0.258
Correlation Coefficient(r)	0.996	0.999
Precision (%Relative Standard Deviation)	0.5981	0.9574
Standard error of estimate	0.0198	0.0061

*Y=mx+c, where X is the concentration in micrograms/ml and Y is absorbance unit.

Table. 2: Assay and Recovery of Alosetron Hydrochloride in Tablet dosage form.

Tablet Formulation	Labelled Amount(Mg)		*Amount Obtained(Mg) By Proposed		% **Recovery By The Proposed Method	
	Method A	Method B	Method A	Method B	Method A	Method B
1.	100mg	100mg	98.8 ±2	97.5 ±2	99.01 ±2	98.4 ±2
2.	100mg	100mg	99.3 ±1	98.8 ±1	99.8 ±1	99.2 ±1
3.	100mg	100mg	100.5 ±1	99.3 ±1	101.2±1	101.17 ±1

*Average of three determination.
**After spiking the sample.

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